(19) World Intellectual Property Organization

International Bureau





(43) International Publication Date 6 May 2005 (06.05.2005)

(10) International Publication Number WO 2005/040377 A3

- (51) International Patent Classification7: C12N 15/11. G06F 19/00, C12N 5/10, A01K 67/027, C12N 15/67, 15/79
- (21) International Application Number:

PCT/EP2004/011974

- (22) International Filing Date: 22 October 2004 (22.10.2004)
- (25) Filing Language:

English

(26) Publication Language: English

(30) Priority Data:

24 October 2003 (24.10.2003) US 60/513,574 04002722.9 6 February 2004 (06.02.2004) EP

- (71) Applicant (for all designated States except US): SELEXIS S.A. [CH/CH]; 18, Chemin des Aulx, CH-1228 Plan-les-Ouates (CH).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): MERMOD, Nicolas [CH/CH]; Route des Deux-Communes 19, CH-1164 Buchillon (CH). GIROD, Pierre Alain [CH/CH]; Ch. du Muguet 15, CH-1007 Lausanne (CH). BUCHER, Philipp [CH/CH]; Avenue de Valmont 18, CH-1010 Lausanne (CH). NGUYEN, Duc-Quang [CH/CH]; Ch. des Etourneaux 24, CH-1162 Saint Prex (CH). CALABRESE, David [CH/CH]; Pl. du Tunnel 4, CH-1005 Lausanne (CH). SAUGY, Damien [CH/CH]; Avenue Mon-Loisir 13, CH-1006 Lausanne (CH). PUTTINI, Stefania [IT/CH]; Avenue Marc-Dufour 50, CH-1007 Lausanne (CH).

- (74) Agent: IPRIS GMBH; Missionsstrasse 24, CH-4055 Basel (CH).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report: 15 September 2005

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: HIGH EFFICIENCY GENE TRANSFER AND EXPRESSION IN MAMMALIAN CELLS BY A MULTIPLE TRANS-FECTION PROCEDURE OF MATRIX ATTACHMENT REGION SEQUENCES

(57) Abstract: The present invention relates to purified and isolated DNA sequences having protein production increasing activity and more specifically to the use of matrix attachment regions (MARs) for increasing protein production activity in a eukaryotic cell. Also disclosed is a method for the identification of said active regions, in particular MAR nucleotide sequences, and the use of these characterized active MAR sequences in a new multiple transfection method.



Internation Population No

a. classification of subject matter IPC 7 C12N15/11 G06F19/00 A01K67/027 C12N15/67 C12N5/10 C12N15/79 According to International Patent Classification (IPC) or to both national classification and IPC **B. FIELDS SEARCHED** Minimum documentation searched (classification system followed by classification symbols) G06F IPC 7 C12N A01K Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, BIOSIS, EMBL, Sequence Search C. DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. DATABASE EMBL [Online]
4 January 2002 (2002-01-04), "Human DNA 1-4,7-9,Χ 35-41 sequence from clone RP4-743D20 on chromosome 1 Contains a novel gene and a CpG island." XP002322943 retrieved from EBI accession no. EM HUM:AL663105 Database accession no. AL663105 the whole document -/--Further documents are listed in the continuation of box C. Patent family members are listed in annex. X Special categories of cited documents. "T" later document published after the international filing date or priority date and not in conflict with the application but "A" document defining the general state of the art which is not considered to be of particular relevance cited to understand the principle or theory underlying the invention "E" earlier document but published on or after the international "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the ad-"O" document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of mailing of the international search report Date of the actual completion of the international search 13-07-2005 1 April 2005 Authorized officer Name and mailing address of the ISA European Patent Office, P B. 5818 Patentlaan 2 NL - 2280 HV Rijswyk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax. (+31-70) 340-3016 Kools, P

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT						
Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.					
ZAHN-ZABAL M ET AL: "Development of stable cell lines for production or regulated expression using matrix attachment regions" JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 87, no. 1, 27 April 2001 (2001-04-27), pages 29-42, XP004231294 ISSN: 0168-1656 the whole document	1-4,7-9, 12-14, 35-61					
WO 02/079447 A (AVIGENICS, INC; RAPP, JEFFREY, C) 10 October 2002 (2002-10-10) cited in the application the whole document	1-4,7-9, 12-14, 35-61					
KWAKS TED H J ET AL: "Identification of anti-repressor elements that confer high and stable protein production in mammalian cells" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 21, no. 5, 20 May 2003 (2003-05-20), pages 553-558, XP002246849 ISSN: 1087-0156 the whole document	1-4,7-9, 12-14, 35-61					
WO 00/32800 A (DOW AGROSCIENCES LLC) 8 June 2000 (2000-06-08) the whole document	12-14					
FRISCH M ET AL: "In silico prediction of scaffold/matrix attachment regions in large genomic sequences" GENOME RESEARCH, COLD SPRING HARBOR LABORATORY PRESS, US, vol. 12, no. 2, February 2002 (2002-02), pages 349-354, XP002257587 ISSN: 1088-9051 the whole document	1-4,7-9, 12-14, 35-61					
SINGH G B ET AL: "Mathematical model to predict regions of chromatin attachment to the nuclear matrix" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 25, no. 7, 1997, pages 1419-1425, XP002273524 ISSN: 0305-1048 cited in the application the whole document	1-4,7-9, 12-14, 35-61					
	ZAHN-ZABAL M ET AL: "Development of stable cell lines for production or regulated expression using matrix attachment regions" JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 87, no. 1, 27 April 2001 (2001-04-27), pages 29-42, XP004231294 ISSN: 0168-1656 the whole document WO 02/079447 A (AVIGENICS, INC; RAPP, JEFFREY, C) 10 October 2002 (2002-10-10) cited in the application the whole document KWAKS TED H J ET AL: "Identification of anti-repressor elements that confer high and stable protein production in mammalian cells" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 21, no. 5, 20 May 2003 (2003-05-20), pages 553-558, XP002246849 ISSN: 1087-0156 the whole document WO 00/32800 A (DOW AGROSCIENCES LLC) 8 June 2000 (2000-06-08) the whole document FRISCH M ET AL: "In silico prediction of scaffold/matrix attachment regions in large genomic sequences" GENOME RESEARCH, COLD SPRING HARBOR LABORATORY PRESS, US, vol. 12, no. 2, February 2002 (2002-02), pages 349-354, XP002257587 ISSN: 1088-9051 the whole document SINGH G B ET AL: "Mathematical model to predict regions of chromatin attachment to the nuclear matrix" NUCLEIC ACIDS RESEARCH, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 25, no. 7, 1997, pages 1419-1425, XP002273524 ISSN: 0305-1048 cited in the application the whole document					

Internatio Application No
PCT/EP2004/011974

	ation) DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	
Category °	Citation of document, with indication, where appropriate, or the relevant passages	Tieroveric to Gaini No.	
A	LEVITSKY VICTOR G ET AL: "Nucleosomal DNA property database" BIOINFORMATICS (OXFORD), vol. 15, no. 7-8, July 1999 (1999-07), pages 582-592, XP002322940 ISSN: 1367-4803 cited in the application the whole document	1-4,7-9, 12-14, 35-61	
Α	ROLLINI PIERRE ET AL: "Identification and characterization of nuclear matrix-attachment regions in the human serpin gene cluster at 14q32.1" NUCLEIC ACIDS RESEARCH, vol. 27, no. 19, 1 October 1999 (1999-10-01), pages 3779-3791, XP002322995 ISSN: 0305-1048 the whole document	1-4,7-9, 12-14, 35-61	
Α	BONNEFOY ELIETTE ET AL: "Specific binding of high-mobility-group I (HMGI) protein and histone H1 to the upstream AT-rich region of the murine beta interferon promoter: HMGI protein acts as a potential antirepressor of the promoter" MOLECULAR AND CELLULAR BIOLOGY, vol. 19, no. 4, April 1999 (1999-04), pages 2803-2816, XP002322941 ISSN: 0270-7306 abstract	2,3	
A	COX GEORGE W ET AL: "Molecular cloning and characterization of a novel mouse macrophage gene that encodes a nuclear protein comprising polyglutamine repeats and interspersing histidines" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 41, 1996, pages 25515-25523, XP002322942 ISSN: 0021-9258 abstract	8	

Information on patent family members

Internation pplication No
PCT/EP2004/011974

Patent document cited in search report	Publication date		Patent family member(s)	Publication date
WO 02079447 A	10-10-2002	US EP WO US EP WO US	2002199214 A1 1478751 A2 02079447 A2 2003140363 A1 1438401 A2 03025146 A2 2003126629 A1	26-12-2002 24-11-2004 10-10-2002 24-07-2003 21-07-2004 27-03-2003 03-07-2003
WO 0032800 A	08-06-2000	AT AU DE DE EP ES JP WO	279524 T 1833100 A 69921180 D1 69921180 T2 1135512 A1 2232190 T3 2002531097 T 0032800 A1	15-10-2004 19-06-2000 18-11-2004 17-02-2005 26-09-2001 16-05-2005 24-09-2002 08-06-2000